

What is claimed is:

1. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, wherein the mutation comprises an amino acid substitution in the Beta Strand 4 portion of the protein, relative  
5 to the wild-type form of the protein, and wherein the mutant GFP protein has one or more of the following characteristics:
  - (a) enhanced emission intensity, relative to wild-type GFP protein from *Renilla reniformis*;
  - (b) a narrower or broader emission spectrum, relative to wild-type GFP protein from  
10 *Renilla reniformis*;
  - (c) a shift in excitation or emission maxima, relative to wild-type GFP protein from *Renilla reniformis*;
  - (d) a shift in maturation rate, relative to wild-type GFP protein from *Renilla reniformis*; and
  - 15 (e) exhibiting less quenching of fluorescence at acidic pH, relative to wild-type GFP protein from *Renilla reniformis*.
2. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, wherein the mutation comprises an amino acid substitution in the loop region of the protein between Beta  
20 Strand 2 and Beta Strand 3, relative to the wild-type form of the protein, and wherein the mutant GFP protein has one or more of the following characteristics:
  - (a) enhanced emission intensity, relative to wild-type GFP protein from *Renilla reniformis*;
  - (b) a narrower or broader emission spectrum, relative to wild-type GFP protein from  
25 *Renilla reniformis*;
  - (c) a shift in excitation or emission maxima, relative to wild-type GFP protein from *Renilla reniformis*;
  - (d) a shift in maturation rate, relative to wild-type GFP protein from *Renilla reniformis*; and
  - 30 (e) exhibiting less quenching of fluorescence at acidic pH, relative to wild-type GFP protein from *Renilla reniformis*.

3. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, wherein the mutation comprises an amino acid substitution in the loop region of the protein between Beta Strand 5 and Beta Strand 6, relative to the wild-type form of the protein, and wherein the mutant GFP protein has one or more of the following characteristics:
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- (a) enhanced emission intensity, relative to wild-type GFP protein from *Renilla reniformis*;
  - (b) a narrower or broader emission spectrum, relative to wild-type GFP protein from *Renilla reniformis*;
  - 10 (c) a shift in excitation or emission maxima, relative to wild-type GFP protein from *Renilla reniformis*;
  - (d) a shift in maturation rate, relative to wild-type GFP protein from *Renilla reniformis*; and
  - (e) exhibiting less quenching of fluorescence at acidic pH, relative to wild-type GFP protein from *Renilla reniformis*.
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4. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, wherein the mutation comprises an amino acid substitution in the region of the protein extending from the beginning of Beta Strand 1 through the end of the loop region between Beta Strands 2 and 3, relative to the wild-type form of the protein, and wherein the mutant GFP protein has one or more of the following characteristics:
- 20
- (a) enhanced emission intensity, relative to wild-type GFP protein from *Renilla reniformis*;
  - (b) a narrower or broader emission spectrum, relative to wild-type GFP protein from *Renilla reniformis*;
  - 25 (c) a shift in excitation or emission maxima, relative to wild-type GFP protein from *Renilla reniformis*;
  - (d) a shift in maturation rate, relative to wild-type GFP protein from *Renilla reniformis*; and
  - (e) exhibiting less quenching of fluorescence at acidic pH, relative to wild-type GFP protein from *Renilla reniformis*.
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5. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, wherein the mutation comprises an amino acid substitution in the region of the protein extending from the beginning of Beta Strand 4 through the end of Beta Strand 6, relative to the wild-type form of the protein, and wherein the mutant GFP protein has one or more of the following characteristics:
- (a) enhanced emission intensity, relative to wild-type GFP protein from *Renilla reniformis*;
  - (b) a narrower or broader emission spectrum, relative to wild-type GFP protein from *Renilla reniformis*;
  - (c) a shift in excitation or emission maxima, relative to wild-type GFP protein from *Renilla reniformis*;
  - (d) a shift in maturation rate, relative to wild-type GFP protein from *Renilla reniformis*; and
  - (e) exhibiting less quenching of fluorescence at acidic pH, relative to wild-type GFP protein from *Renilla reniformis*.
6. A polynucleotide encoding mutant *Renilla reniformis* Green Fluorescent Protein (GFP) of claims 1-5.
7. The polynucleotide of claim 6, said polynucleotide being humanized.
8. A vector comprising the polynucleotide of claim 7.
9. A host cell containing the vector of claim 8.
10. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, selected from the group consisting of:
- (a) the amino acid sequence of mutant GM1;
  - (b) the amino acid sequence of mutant GM2;
  - (c) the amino acid sequence of mutant GM3;

- (d) the amino acid sequence of mutant GM4;  
(e) the amino acid sequence of mutant GM6;  
(f) the amino acid sequence of mutant T1;  
(g) the amino acid sequence of mutant T6;  
5 (h) the amino acid sequence of mutant T8;  
(i) the amino acid sequence of mutant T11;  
(j) the amino acid sequence of mutant T12;  
(k) the amino acid sequence of mutant T13;  
(l) the amino acid sequence of mutant T14;  
10 (m) the amino acid sequence of mutant T15; and  
(n) the amino acid sequence of mutant T17.
11. A polynucleotide encoding a mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, selected from the group consisting of:
- 15 (a) a polynucleotide encoding the amino acid sequence of mutant GM1;  
(b) a polynucleotide encoding the amino acid sequence of mutant GM2;  
(c) a polynucleotide encoding the amino acid sequence of mutant GM3;  
(d) a polynucleotide encoding the amino acid sequence of mutant GM4;  
(e) a polynucleotide encoding the amino acid sequence of mutant GM6;  
20 (f) a polynucleotide encoding the amino acid sequence of mutant T1;  
(g) a polynucleotide encoding the amino acid sequence of mutant T6;  
(h) a polynucleotide encoding the amino acid sequence of mutant T8;  
(i) a polynucleotide encoding the amino acid sequence of mutant T11;  
(j) a polynucleotide encoding the amino acid sequence of mutant T12;  
25 (k) a polynucleotide encoding the amino acid sequence of mutant T13;  
(l) a polynucleotide encoding the amino acid sequence of mutant T14;  
(m) a polynucleotide encoding the amino acid sequence of mutant T15; and  
(n) a polynucleotide encoding the amino acid sequence of mutant T17.
- 30 12. The polynucleotide of claim 11, said polynucleotide being humanized.

13. A vector comprising the polynucleotide of claim 12.
14. A host cell containing the vector of claim 13.
- 5 15. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, selected from the group consisting of:
- (a) the amino acid sequence of SEQ ID NO:34;
  - (b) the amino acid sequence of SEQ ID NO:36;
  - (c) the amino acid sequence of SEQ ID NO:38;
  - 10 (d) the amino acid sequence of SEQ ID NO:40;
  - (e) the amino acid sequence of SEQ ID NO:42;
  - (f) the amino acid sequence of SEQ ID NO:44;
  - (g) the amino acid sequence of SEQ ID NO:46;
  - (h) the amino acid sequence of SEQ ID NO:48;
  - 15 (i) the amino acid sequence of SEQ ID NO:50;
  - (j) the amino acid sequence of SEQ ID NO:52;
  - (k) the amino acid sequence of SEQ ID NO:54;
  - (l) the amino acid sequence of SEQ ID NO:56;
  - (m) the amino acid sequence of SEQ ID NO:58; and
  - 20 (n) the amino acid sequence of SEQ ID NO:60.
16. A polynucleotide encoding a mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, selected from the group consisting of:
- (a) the polynucleotide sequence of SEQ ID NO:33;
  - 25 (b) the polynucleotide sequence of SEQ ID NO:35;
  - (c) the polynucleotide sequence of SEQ ID NO:37;
  - (d) the polynucleotide sequence of SEQ ID NO:39;
  - (e) the polynucleotide sequence of SEQ ID NO:41;
  - (f) the polynucleotide sequence of SEQ ID NO:43;
  - 30 (g) the polynucleotide sequence of SEQ ID NO:45;
  - (h) the polynucleotide sequence of SEQ ID NO:47;

- (i) the polynucleotide sequence of SEQ ID NO:49;
  - (j) the polynucleotide sequence of SEQ ID NO:51;
  - (k) the polynucleotide sequence of SEQ ID NO:53;
  - (l) the polynucleotide sequence of SEQ ID NO:55;
  - 5 (m) the polynucleotide sequence of SEQ ID NO:57; and
  - (n) the polynucleotide sequence of SEQ ID NO:59.
17. The polynucleotide of claim 16, said polynucleotide being humanized.
- 10 18. A vector comprising the polynucleotide of claim 17.
19. A host cell containing the vector of claim 18.
20. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, selected from the
- 15 group consisting of:
- (a) the amino acid sequence of SEQ ID NO:4;
  - (b) the amino acid sequence of SEQ ID NO:6;
  - (c) the amino acid sequence of SEQ ID NO:8;
  - (d) the amino acid sequence of SEQ ID NO:10;
  - 20 (e) the amino acid sequence of SEQ ID NO:12;
  - (f) the amino acid sequence of SEQ ID NO:14;
  - (g) the amino acid sequence of SEQ ID NO:16;
  - (h) the amino acid sequence of SEQ ID NO:18;
  - (i) the amino acid sequence of SEQ ID NO:20;
  - 25 (j) the amino acid sequence of SEQ ID NO:22;
  - (k) the amino acid sequence of SEQ ID NO:24;
  - (l) the amino acid sequence of SEQ ID NO:26;
  - (m) the amino acid sequence of SEQ ID NO:28; and
  - (n) the amino acid sequence of SEQ ID NO:30.

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21. A polynucleotide encoding a mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, selected from the group consisting of:
- (a) the polynucleotide sequence of SEQ ID NO:3;
  - (b) the polynucleotide sequence of SEQ ID NO:5;
  - 5 (c) the polynucleotide sequence of SEQ ID NO:7;
  - (d) the polynucleotide sequence of SEQ ID NO:9;
  - (e) the polynucleotide sequence of SEQ ID NO:11;
  - (f) the polynucleotide sequence of SEQ ID NO:13;
  - (g) the polynucleotide sequence of SEQ ID NO:15;
  - 10 (h) the polynucleotide sequence of SEQ ID NO:17;
  - (i) the polynucleotide sequence of SEQ ID NO:19;
  - (j) the polynucleotide sequence of SEQ ID NO:21;
  - (k) the polynucleotide sequence of SEQ ID NO:23;
  - (l) the polynucleotide sequence of SEQ ID NO:25;
  - 15 (m) the polynucleotide sequence of SEQ ID NO:27; and
  - (n) the polynucleotide sequence of SEQ ID NO:29.
22. A vector comprising the polynucleotide of claim 21.
- 20 23. A host cell containing the vector of claim 22.
24. A method of producing mutant *Renilla reniformis* GFP comprising the steps of:
- (a) culturing a cell containing a recombinant vector comprising a wild type or humanized polynucleotide sequence encoding mutant *Renilla reniformis* GFP under conditions where the mutant *Renilla reniformis* GFP protein is expressed; and
  - 25 (b) isolating said mutant *Renilla reniformis* GFP protein from said cell; thereby producing mutant *Renilla reniformis* GFP.
- 30 25. A method of producing a *Renilla reniformis* fusion protein, said method comprising the steps of: culturing a cell containing a polynucleotide sequence encoding said polypeptide

of interest linked with a humanized polynucleotide encoding mutant *Renilla reniformis* GFP wherein the linked polynucleotide sequences are fused in frame, under conditions where the mutant *Renilla reniformis* GFP protein is expressed.

- 5     26.     A method of determining the location of a polypeptide of interest in a cell, said method comprising determining the location of the fusion protein of claim 25.
27.     A method of identifying a cell into which a recombinant vector has been introduced, said method comprising the steps of:
- 10     (a)     providing a cell containing a recombinant vector comprising a humanized polynucleotide which encodes mutant *Renilla reniformis* GFP, wherein said cell permits expression of said humanized polynucleotide;
- (b)     illuminating said population with light within the excitation spectrum of mutant *Renilla reniformis* GFP; and
- 15     (c)     detecting fluorescence in the emission spectrum of mutant *Renilla reniformis* GFP in said population, where detection of fluorescence in the cell indicates that the recombinant vector has been introduced into the cell;
- thereby identifying a cell into which said recombinant vector has been introduced.
- 20     28.     The method of claim 27, wherein said GFP is expressed as a fusion polypeptide.
29.     The method of claim 27, wherein said GFP is expressed as a distinct polypeptide.
30.     The method of claim 27, wherein said cells are identified by FACS analysis.
- 25     31.     A method of detecting the activity of a transcriptional regulatory sequence, said method comprising the steps of:
- (a)     culturing a cell containing a nucleic acid sequence comprising said transcriptional regulatory sequence operably linked to a humanized nucleic acid sequence
- 30     encoding mutant *Renilla reniformis* GFP to form a reporter construct, under conditions where the mutant *Renilla reniformis* GFP is expressed; and



- (b) detecting mutant *Renilla reniformis* GFP fluorescence in said cell, wherein detection of fluorescence indicates activity of said transcriptional regulatory sequence;

thereby detecting the activity of a transcriptional regulatory sequence.

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32. A method of detecting the presence of a modulator of a transcriptional regulatory sequence, said method comprising the steps of:

- (a) culturing a cell containing a nucleic acid sequence comprising said transcriptional regulatory sequence operably linked to a humanized nucleic acid sequence encoding mutant *Renilla reniformis* GFP to form a reporter construct, under conditions where the mutant *Renilla reniformis* GFP is expressed; and
- (b) detecting mutant *Renilla reniformis* GFP fluorescence in said cell, wherein said fluorescence indicates the presence of said modulator;

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thereby detecting the presence of a modulator of a transcriptional regulatory sequence.

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33. A method of screening for an inhibitor of a transcriptional regulatory sequence, said method comprising the steps of:

- (a) culturing a cell containing a nucleic acid sequence comprising said transcriptional regulatory sequence operably linked to a humanized nucleic acid sequence encoding mutant *Renilla reniformis* GFP to form a reporter construct, under conditions where the mutant *Renilla reniformis* GFP is expressed;
- (b) contacting said cell with a candidate inhibitor of said transcriptional regulatory sequence; and
- (c) detecting mutant *Renilla reniformis* GFP fluorescence in said cell, wherein a decrease in said fluorescence relative to that detected in the absence of said candidate inhibitor indicates that said candidate inhibitor inhibits the activity of said transcriptional regulatory sequence.

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34. A method of producing a fluorescent molecular weight marker, said method comprising the steps of:

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- 5 (a) culturing a cell containing a humanized nucleic acid sequence encoding mutant *Renilla reniformis* GFP linked in frame to a nucleic acid sequence encoding a polypeptide of known relative molecular weight such that said linked molecules encode a fusion polypeptide, under conditions where the mutant *Renilla reniformis* GFP is expressed;
- (b) isolating said fusion polypeptide from said cell, wherein said fusion polypeptide is a relative molecular weight marker.
- 10 35. The method of claims 24, 25, 27 or 31-34, wherein said cell is a mammalian cell.
36. The method of claims 24, 25, 27 or 31-34, wherein said cell is a human cell.
- 15 37. The method of claims 24, 25, 27 or 31-34, wherein said mutant *Renilla reniformis* GFP is selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28 and SEQ ID NO:30.
- 20 38. The method of claims 24, 25, 27 or 31-34, wherein said nucleic acid sequence encoding mutant *Renilla reniformis* GFP is selected from the group consisting of: SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27 and SEQ ID NO:29.
- 25 39. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, wherein the mutation comprises an amino acid substitution in one of the following regions of the protein, relative to the wild-type form of the protein:
- 30 (a) the Beta Strand 4 region of the protein;
- (b) the loop region of the protein between Beta Strand 2 and Beta Strand 3;
- (c) the loop region of the protein between Beta Strand 5 and Beta Strand 6;

- (d) the region of the protein extending from the beginning of Beta Strand 1 through the end of the loop region between Beta Strands 2 and 3; and
- (e) the region of the protein extending from the beginning of Beta Strand 4 through the end of Beta Strand 6;

5 and wherein the mutant GFP protein has one or more of the following characteristics:

- (r) exhibiting less quenching over a broad pH range, relative to wild-type GFP protein from *Renilla reniformis*;
- (s) exhibiting greater resistance to one or more of the following: proteases, solvents, detergents and chaotropic agents; and
- 10 (t) exhibiting reduced tendency to oligomerize.

40. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, wherein the mutation comprises an amino acid substitution at one or more of the following residues:

- (a) F43;
- 15 (b) E120;
- (c) L101; and
- (d) Y103.